5 Microbial Metabolism

CHAPTER SUMMARY

Basic Chemical Reactions Underlying Metabolism (pp. 124–133)

Catabolism and Anabolism

Metabolism is the sum of complex biochemical reactions within an organism, including **catabolic reactions** that break down nutrient molecules and release energy stored in ATP molecules (exergonic) and **anabolic reactions** that synthesize macromolecules and use ATP energy (endergonic).

Enzymes catabolize nutrients into **precursor metabolites**, which are rearranged by polymerization reactions to form macromolecules. Cells grow as they assemble these large molecules into cell parts. Reproduction usually occurs when the cell has doubled in size.

Oxidation and Reduction Reactions

Oxidation-reduction (redox) reactions involve the transfer of electrons. These reactions always occur simultaneously because an electron gained by one molecule is donated by another molecule. The electron acceptor is said to be **reduced**. The molecule that loses an electron is **oxidized**. If the electron is part of a hydrogen atom, the reaction is called a dehydrogenation reaction.

Three electron carrier molecules that are often required in metabolic pathways are **nicotinamide adenine dinucleotide** (NAD^+), **nicotinamide adenine dinucleotide phosphate** ($NADP^+$), **and flavine adenine dinucleotide** (FAD).

ATP Production and Energy Storage

Energy from the chemical bonds of nutrients is concentrated in the high-energy phosphate bonds of ATP. **Substrate-level phosphorylation** describes the transfer of phosphate from a phosphorylated organic nutrient to ADP to form ATP. **Oxidative phosphorylation** phosphorylates ADP using inorganic phosphate and energy from respiration. **Photophosphorylation** is the phosphorylation of ADP with inorganic phosphate using energy from light. There is a cyclical conversion of ATP from ADP and back with the gain and loss of phosphate.

The Roles of Enzymes in Metabolism

Catalysts increase reaction rates of chemical reactions but are not permanently changed in the process. **Enzymes**, organic catalysts, are often named for their **substrates**, which are the chemicals they cause to react. Substrates fit onto the specifically shaped **active sites** of enzymes.

Naming and Classifying Enzymes

Enzymes are classified into six categories based on their mode of action: *hydrolases* add hydrogen and hydroxide from the hydrolysis of water to split larger molecules

into smaller ones; *lyases* split molecules without using water; *isomerases* form isomeric compounds; *ligases* or *polymerases* join molecules; *oxidoreductases* oxidize or reduce; and *transferases* transfer functional groups.

The Makeup of Enzymes

Many protein enzymes are complete in themselves. Other enzymes are composed of **apoenzymes**—a protein portion—and one or more nonprotein **cofactors**. Inorganic cofactors include ions such as iron, magnesium, zinc, or copper. Organic cofactors are made from vitamins and include NAD^+, $NADP^+$, and FAD. Organic cofactors are also called **coenzymes**. The combination of both apoenzyme and its cofactors is a **holoenzyme**.

RNA molecules functioning as enzymes are called **ribozymes**. Ribozymes process RNA molecules in eukaryotes. Ribosomal enzymes catalyze the actual protein synthesis reactions of ribosomes; thus, ribozymes make protein enzymes.

Enzyme Activity

Activation energy is the amount of energy required to initiate a chemical reaction. Activation energy may be supplied by heat, but high temperatures are not compatible with life; therefore, enzymes are required to lower the activation energy needed. The complementary shapes of active sites of enzymes and their substrates determine enzyme-substrate specificity. In catabolism, an enzyme binds to a substrate, forming an enzyme-substrate *complex*, the bonds within the substrate are broken, the enzyme separates from the two new products, and the enzyme is released to act again.

Enzymes may be **denatured** by physical and chemical factors such as temperature and pH, which change their shape and thus their ability to bond. The change may be reversible or permanent.

The rate of enzymatic activity is also affected by the concentrations of substrate and enzyme. Enzyme activity proceeds at a rate proportional to the concentration of substrate molecules until all the active sites on the enzymes are filled to saturation.

Enzyme activity may be blocked by **competitive inhibitors**, which block but do not denature active sites. In **allosteric inhibition**, **noncompetitive inhibitors** attach to an allosteric site on an enzyme distorting the active site and halting enzymatic activity. In *excitatory allosteric control,* the change in the shape of the active site activates an inactive enzyme.

Feedback inhibition (**negative feedback**) occurs when the final product of a series of reactions is an allosteric inhibitor of some previous step in the series. Thus accumulation of the end product "feeds back" a stop signal to the process.

Carbohydrate Catabolism (pp. 133–147)

Glycolysis

Glycolysis (the *Embden-Meyerhof pathway*) involves the splitting of a glucose molecule in a series of ten steps which ultimately results in two molecules of pyruvic acid and a net gain of two ATP and two NADH molecules. The ten steps of glycolysis can be divided into three stages: energy-investment, lysis, and energy-conserving.

Alternatives to Glycolysis

The **pentose phosphate** and **Entner-Doudoroff pathways** are alternative pathways for the catabolism of glucose, but they yield fewer ATP molecules than does the Embden-Meyerhof pathway. However, they produce precursor metabolites not

produced in glycolysis. The pentose phosphate pathway produces metabolites used in synthesis of nucleotides, amino acids, and glucose by photosynthesis. The Entner-Doudoroff pathway, used by only a few bacteria, uses different enzymes and yields precursor metabolites and NADPH, which is not produced by the Embden-Meyerhof or pentose phosphate pathways.

Cellular Respiration

Cellular respiration is a three-stage metabolic process that involves oxidation of substrate molecules and production of ATP. The stages of respiration are: synthesis of acetyl-CoA, Krebs cycle, and electron transport.

Synthesis of Acetyl-CoA and the Krebs Cycle

Acetyl-Coenzyme A (**acetyl-CoA**) is formed when two carbons from pyruvic acid join coenzyme A. Two molecules of acetyl-CoA, two molecules of CO_2, and two molecules of NADH are produced. Acetyl-CoA enters the **Krebs cycle**, a series of enzymatic steps that transfer energy and electrons from acetyl-CoA to coenzymes NAD^+ and FAD. For every two molecules of acetyl CoA that enter the Krebs cycle, two molecules of ATP, six molecules of NADH and two molecules of $FADH_2$ are formed.

Electron Transport

The **electron transport chain** is a series of redox reactions that passes electrons from one membrane-bound carrier to another and then to a *final electron acceptor*. The energy from these electrons is used to pump protons (H^+) across the membrane. Ultimately ATP is synthesized.

The four categories of carrier molecules in the electron transport system are flavoproteins, ubiquinones, proteins containing heavy metal, and cytochromes.

Aerobes use oxygen atoms as final electron acceptors in the electron transport chain in a process known as **aerobic respiration**, whereas anaerobes use other inorganic molecules such as sulfate, nitrate, and carbonate as final electron acceptors in **anaerobic respiration**.

Chemiosmosis

Chemiosmosis is a mechanism in which the flow of ions down an *electrochemical gradient* across a membrane is used to synthesize ATP. For example, energy released during the redox reactions of electron transport is used to pump protons across a membrane, creating a proton gradient.

A **proton gradient** is an electrochemical gradient of protons that has potential energy known as a *proton motive force*. When protons flow down their electrochemical gradient through protein channels called ATPases, ATP is synthesized. **ATP synthases** (ATPases) are enzymes that synthesize ATP by oxidative phosphorylation and photophosphorylation.

About 34 ATP molecules are synthesized per pair of electrons traveling an electron transport chain. Thus, there is a theoretical net yield of 38 ATP molecules from the aerobic respiration of 1 molecule of glucose via glycolysis (4 molecules of ATP produced minus 2 molecules of ATP used), Krebs cycle (2 molecules of ATP produced), and electron transport chain (34 molecules of ATP produced).

Fermentation

Fermentation is the partial oxidation of sugar to release energy using an organic molecule within the cell as electron acceptor. In lactic acid fermentation, NADH reduces pyruvic acid from glycolysis to form lactic acid. In alcohol fermentation, pyruvic acid

undergoes decarboxylation (CO_2 is given off) and reduction by NADH to form ethanol. Some fermentation products are useful to health and industry while some are harmful.

Other Catabolic Pathways (pp. 147–149)

Lipid Catabolism

Fats are catabolized by lipases that break the glycerol-fatty acid bonds via hydrolysis. Glycerol is converted to DHAP to be catabolized by glycolysis and the Krebs cycle. Fatty acids are catabolized by **beta-oxidation** reactions that form acetyl-CoA and generate NADH and $FADH_2$.

Protein Catabolism

Protein catabolism by prokaryotes involves **protease** enzymes secreted to digest large proteins outside their cell walls. The resulting amino acids move into the cell and are used in anabolism or **deaminated** to produce substrates for the Krebs cycle.

Photosynthesis (pp. 149–154)

Chemicals and Structures

Photosynthesis is a process in which light energy is captured by pigment molecules (called **chlorophylls**) and transferred to ATP and metabolites. **Photosystems**, photosystem I (PS I) and photosystem II (PS II), are networks of chlorophyll molecules and other pigments held within a protein matrix in membranes called **thylakoids**.

Prokaryotic thylakoids are infoldings of the cytoplasmic membrane whereas eukaryotic thylakoids are infoldings of the inner membranes of chloroplasts. Stacks of thylakoids within chloroplasts are called *grana*.

Light-Dependent Reactions

The light absorption and redox reactions of photosynthesis are classified as **light-dependent reactions** (*light reactions*) and **light-independent reactions** (*dark reactions*). The latter synthesize glucose from carbon dioxide and water regardless of light conditions.

A **reaction center chlorophyll** is a special chlorophyll molecule of photosystem I, which is excited by transferred energy absorbed by pigment molecules elsewhere in the photosystem. Excited electrons from the reaction center are passed to an acceptor of an electron transport chain, protons are pumped across the membrane, a proton motive force is created, and ATP is generated in a process called *photophosphorylation.*

Cyclic Photophosphorylation

In **cyclic photophosphorylation** electrons return to the original reaction center chlorophyll after passing down the electron transport chain. The resulting proton gradient produces ATP by chemiosmosis.

Noncyclic photophosphorylation

In **noncyclic photophosphorylation**, photosystem II works with photosystem I, and the electrons are used to reduce $NADP^+$ to NADPH. Therefore, in noncyclic photophosphorylation, a cell must constantly replenish electrons to PS II. In *oxygenic* organisms, the electrons come from H_2O. In *anoxygenic* organisms, the electrons come from inorganic compounds such as H_2S.

Light-Independent Reactions

ATP and NADPH from the light-dependent reactions drive the synthesis of glucose in the light-independent pathway of photosynthesis. The **Calvin-Benson cycle** of the light-independent pathway occurs in three steps: **carbon fixation** in which CO_2 is reduced; reduction by NADPH to form molecules of G3P, which join to form glucose; and regeneration of RuBP to continue the cycle.

Other Anabolic Pathways (pp. 154–158)

Because anabolic reactions are synthesis reactions, they require energy and metabolites, both of which are often the products of catabolic reactions. **Amphibolic reactions** are metabolic reactions that can proceed toward catabolism or toward anabolism depending on the needs of the cell. Examples are found in the biosynthesis of carbohydrates, lipids, amino acids, and nucleotides.

Carbohydrate Biosynthesis

Gluconeogenesis refers to metabolic pathways that produce sugars, starch, cellulose, glycogen, peptidoglycan, etc., from noncarbohydrate precursors such as amino acids, glycerol, and fatty acids.

Lipid Biosynthesis

Lipids are synthesized by a variety of routes. Steroids result from complex pathways involving polymerizations and isomerizations of sugar and amino acid metabolites. Fat is synthesized from glycerol and three molecules of fatty acid—a reverse of the catabolic reaction.

Amino Acid Biosynthesis

Amino acids are synthesized by **amination**, a process in which the amine group from ammonia is added to a precursor metabolite, and by **transamination**, a reversible reaction in which an amine group is transferred from one amino acid to another by the action of enzymes using coenzyme *pyridoxal phosphate*.

Nucleotide Biosynthesis

Nucleotides are produced from precursor metabolites derived from glycolysis and the Krebs cycle: ribose and deoxyribose from ribose-5 phosphate, phosphate from ATP, and purines and pyrimidines from the amino acids glutamine and aspartic acid.

Integration and Regulation of Metabolic Functions (pp. 158–161)

Energy released in catabolic reactions is used to drive anabolic reactions. Catabolic pathways produce metabolites to use as substrates for anabolic reactions.

The pathways of cellular metabolism can be categorized into three groups: pathways synthesizing macromolecules (proteins, nucleic acids, polysaccharides, and lipids), intermediate pathways, and pathways that produce ATP and precursor molecules (glycolysis, Krebs cycle, pentose phosphate pathway, and Entner-Doudoroff pathway).

Cells use a variety of mechanisms to regulate metabolism including *control of gene expression*, which controls enzyme production needed for metabolic path-

ways, and *control of metabolic expression* in which the cells control enzymes that have been produced.

KEY THEMES

So far we have looked at history, basic chemistry, microbial structure and function, and methodologies for studying microbes. One common thread running through all of these previous chapters is microbial metabolism. Microbiologists have been concerned with metabolic function since the early days of microbiology. Chemistry is the reason metabolism works, and much of the structure and function of the cell is given over to ensuring metabolism continues. Many of the methods for identifying microbes depend on metabolic differences between organisms. As you study this chapter, keep this one fundamental concept in mind:

- *The survival of all life depends on metabolism:* Catabolism drives anabolism which is the foundation for new life; energy is derived from and used by these processes. Life ends when metabolism stops, for microbes and for us. Knowing how microbes work helps us to understand how we work, and helps keep us alive.

QUESTIONS FOR FURTHER REVIEW

Answers to these questions can be found in the answer section at the back of this study guide. Refer to the answers only after you have attempted to solve the questions on your own.

Multiple Choice

1. The ultimate goal of metabolism is to:
   a. Allow the organism to grow
   b. Allow the organism to reproduce
   c. Allow the organism to respond to the environment
   d. Allow the organism to move about its environment
2. Any given catabolic pathway can produce:
   a. ATP only
   b. Metabolites only
   c. Both ATP and metabolites
   d. All of the above
3. In the reaction $NAD^+ \rightarrow NADH$, oxidation is occurring by:
   a. Loss of an electron
   b. Loss of a hydrogen atom
   c. Gain of a hydrogen atom
   d. As written, the reaction shows reduction, not oxidation
4. Which of the following sees the production of energy from respiration?
   a. Substrate-level phosphorylation
   b. Oxidative phosphorylation
   c. Photophosphorylation
   d. All of the above
5. The chemical reactions that sustain life depend on which of the following to keep them going?
   a. High concentrations of reactants
   b. High temperatures
   c. Random collisions between reactants
   d. Organic catalysts called enzymes

6. The movement of the phosphate from glucose 6-phosphate to ADP would be performed by what type of enzyme?
   a. Isomerase
   b. Transferase
   c. Oxidoreductase
   d. Hydrolase

7. Cofactors are:
   a. Inorganic ions
   b. Small organic molecules
   c. Part of a holoenzyme
   d. All of the above

8. Which of the following methods is not used by a cell to regulate enzyme function?
   a. Synthesis of enzymes only when needed
   b. Sequestering of enzymes in organelles
   c. Feedback inhibition
   d. All of the above are methods of regulating enzyme function

9. Competitive inhibitors and noncompetitive inhibitors are similar in that:
   a. Both bind the active site of an enzyme
   b. Both bind away from the active site of an enzyme
   c. Both can turn enzymes off
   d. Both can turn enzymes on

10. The primary energy source that is oxidized to allow anabolism to occur is:
    a. Carbohydrates
    b. Lipids
    c. Proteins
    d. Nucleic acids

11. Which of the following is common to both respiration and fermentation?
    a. Electron transport chain
    b. Krebs cycle
    c. Glycolysis
    d. Beta-oxidation

12. Substrate-level phosphorylation can best be described as:
    a. The direct transfer of phosphate from ATP to substrates
    b. The direct transfer of phosphate from ADP to substrates
    c. The direct transfer of phosphate between ADP and ATP molecules
    d. The direct transfer of phosphate from one substrate to another

13. The end product of glycolysis is:
    a. 2 phosphoenopyruvic acids
    b. 2 pyruvic acids
    c. 2 acetyl CoAs
    d. 2 glyceraldehyde 3-phosphates

14. The pentose phosphate pathway is primarily used to:
    a. Serve as a backup in case the cell can no longer use glycolysis
    b. Produce more ATP than glycolysis when the cell needs more energy quickly
    c. Produce precursor metabolites used in anabolism
    d. Produce NADH which is not made by glycolysis

15. The pentose phosphate pathway provides what essential metabolite to photosynthesis?
    a. Glucose 6-phosphate
    b. Ribulose 5-phosphate
    c. Glyceraldehyde 3-phosphate
    d. Pyruvic acid

16. The final oxidation product of glucose following cellular respiration is:
    a. Pyruvic acid
    b. Carbon dioxide
    c. Acetyl CoA
    d. ATP

17. Which system is responsible for producing the most ATP?
    a. Glycolysis
    b. Krebs cycle
    c. Electron transport chain
    d. All produce equal amounts of ATP

18. Coenzyme Q is an example of which of the four categories of carrier molecules used in the electron transport chain?
    a. Flavoproteins
    b. Ubiquinones
    c. Metaloproteins
    d. Cytochromes

19. Oxidative phosphorylation differs from substrate-level phosphorylation in that:
    a. It generates ATP and substrate-level phosphorylation does not
    b. It generates ATP by using a proton motive force
    c. It is not used to generate ATP
    d. It produces less ATP than substrate-level phosphorylation

20. Which of the following could be a final electron acceptor in fermentation?
    a. Oxygen
    b. Sulfate
    c. Organic molecules
    d. All of the above can be used

21. The major purpose of fermentation is to:
    a. Produce ATP from ADP
    b. Produce NAD^+ from NADH
    c. Produce alcohol
    d. Take the place of glycolysis

22. Which of the following is true of beta-oxidation?
    a. It creates acetyl CoA from breaking down the hydrocarbon portion of lipids
    b. It produces DHAP for glycolysis
    c. It generates NADH for the electron transport chain
    d. All of the above are true of beta-oxidation

23. Bacteriochlorophylls are found in which photosynthetic organism?
    a. Algae
    b. Cyanobacteria
    c. Green and purple bacteria
    d. Archaea

24. During photosynthesis, glucose is synthesized from carbon dioxide and water using:
    a. Light-dependent reactions
    b. Light-independent reactions
    c. The Calvin-Benson cycle
    d. Both b and c

25. ATP is produced in photosynthesis by:
    a. Substrate-level phosphorylation
    b. Chemiosmosis
    c. Aerobic respiration
    d. Anaerobic respiration

26. Which of the following pathways does not contribute precursor metabolites to the synthesis of amino acids?
    a. Glycolysis
    b. Krebs cycle
    c. Pentose phosphate pathway
    d. Gluconeogenesis

27. The majority of metabolic pathways are:
    a. Amphibolic
    b. Anabolic
    c. Catabolic
    d. Few pathways are amphibolic, but there are roughly equal numbers of catabolic and anabolic pathways

Fill in the Blanks

1. Catabolism provides _____ and _____ to fuel anabolism. In general, catabolic pathways are _____

(exergonic/endergonic), which means they _____ (release/require) energy.

2. In redox reactions, electron _____ give electrons to another molecule and are said to be _____. The molecule that accepts the electron is an electron _____ and is said to be _____.
3. The three important electron carriers found in metabolic pathways are _____, _____, and _____.
4. Enzyme inhibitors can either be _____ or _____. This type of inhibition, _____, can sometimes be overcome by simply adding more of the enzyme's substrate to the reaction.
5. Glucose is the favored "input" for catabolism. It is broken down (catabolized) either by _____ or by _____ (name the general metabolic processes).
6. Two pathways can take the place of glycolysis, at least partially. These pathways are _____ and _____. All three pathways produce _____ that can be sent to the Krebs cycle during cellular respiration.
7. Indicate where the following pathways occur in prokaryotic and eukaryotic cells:

Pathway	*Prokaryote*	*Eukaryote*
Glycolysis		
Krebs Cycle		
Electron Transport Chain		
Photosynthesis		

8. The electron transport chain moves electrons from acceptor to acceptor, causing the transfer of _____ across the membrane to establish a _____; ATP is then generated by the process of _____.
9. The final electron acceptor in aerobic respiration is _____ while the final electron acceptor in anaerobic respiration is generally _____.
10. During the catabolism of proteins, _____ cleave the proteins into amino acids which have their amino groups removed by _____ prior to being funneled into the _____ to be recycled.
11. _____ photophosphorylation uses only _____ which serves as the initial electron donor and final electron acceptor. _____ photophosphorylation uses _____ and _____.
12. Glycolysis and gluconeogenesis are examples of _____ pathways.

Matching

(Use the key on the right to match the pathway with the molecules generated. Each letter may be used more than once and each molecule may have more than one answer.)

1. ____ Glyceraldehyde 3-phosphate
2. ____ ATP
3. ____ NADH
4. ____ NAD^+
5. ____ NADPH
6. ____ Glucose
7. ____ CO_2
8. ____ Ribose 5-phosphate
9. ____ Pyruvic acid
10. ____ Glucose 6-phosphate

A. Glycolysis
B. Pentose Phosphate Pathway
C. Entner-Doudoroff Pathway
D. Calvin-Benson Cycle
E. Krebs Cycle
F. Electron Transport Chain
G. Gluconeogenesis

Short-Answer Questions for Thought and Review

1. Explain the process of allosteric inhibition. How can this type of inhibition be overcome?

2. Fermentation produces much less energy than respiration but it is used by many cells. Why is fermentation necessary for the cells that perform it?

3. Why must the rate of anabolism be linked to the rate of catabolism in the cell? For example, what would happen if catabolism proceeded at a much slower rate than anabolism?

4. Write a complete chemical equation for one of the four redox reactions that occur in the Krebs cycle. Indicate which molecules are electron donors, electron acceptors, which are oxidized, and which are reduced.

Critical Thinking

1. What advantage does a cell that can make everything it needs from precursor metabolites have over a cell that must acquire some of these metabolites from nutrient sources in the environment? What is the disadvantage? Why is it best to be able to do both?
2. Glycolysis and gluconeogenesis do opposite reactions but use many of the same enzymes. How does the cell keep the process it needs going in one direction in a situation like this? For example, how do you make glucose with the same pathway that wants to break it down? Relate your answer to the pathways themselves and to what you have learned about the regulation of enzymes.
3. Explain as specifically as you can what happens to ATP production when a bacterial cell growing in an oxygenated environment suddenly finds itself in an anaerobic environment.

Concept Building Questions

1. A microbe living in the environment will catabolize proteins, bring the parts into the cell and, through anabolism, rebuild new proteins. Since many of the proteins used in the cell are the same across species, why can't microbes simply import whole proteins and not expend ATP to make them? Relate your answer to microbial structure which was discussed in Chapter 3.

2. Enzymes reduce the activation energy needed to allow chemical reactions to occur by creating enzyme-substrate complexes that are conducive to the reaction. What types of bonds can form between an enzyme and substrate when forming this complex that will help reduce activation energy but still allow the reaction to occur? What types of bonds *can't* form between an enzyme and substrate so as to prevent interference with the reaction? If these "wrong" bonds formed in the complex, what could happen to the reaction and why?